Institute for Reference Materials and Measurements

ERM European Reference Materials

CERTIFICATION REPORT

The Certification of the Mass Fractions of Proximates and Essential Elements in Lyophilised Pork Muscle

Certified Reference Material ERM®-BB384

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The Certification of the Mass Fractions of Proximates and Essential Elements in Lyophilised Pork Muscle

Certified Reference Material ERM®-BB384

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Summary

This report describes the preparation of the lyophilised pork muscle matrix reference material ERM®-BB384 and the certification of the contents (mass fractions) of three proximates and four essential elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the materials, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation. The certified values and their uncertainties are listed in Table I:

Table I: Certified mass fractions of proximates and essential elements and their uncertainties in lyophilised pork muscle (ERM®-BB384)

Proximates and essential elements	Certified value [1)]	Uncertainty [2)]	Number of accepted sets of results
Kjeldahl nitrogen [3)]	14.2 g/100 g	0.4 g/100 g	9
Total fat [4)]	8.99 g/100 g	0.20 g/100 g	10
Ash [5)]	4.51 g/100 g	0.19 g/100 g	9
Na	1.86 mg/g	0.15 mg/g	10
Mg	1.03 mg/g	0.04 mg/g	9
Ca	0.164 mg/g	0.021 mg/g	8
P	8.7 mg/g	0.5 mg/g	9

1) These values are related to dry mass and are based on the unweighted mean of accepted results
2) The uncertainties are the expanded uncertainties ($k = 2$) of the certified values
3) Protein can be derived by multiplying Kjeldahl nitrogen with an appropriate factor (e.g. see ISO 937 [2])
4) Total fat determined after acid hydrolysis, solvent extraction and subsequent gravimetry
5) Ashing at 550 °C ± 25 °C

The assigned values and their uncertainties are based on minimum sample intakes varying from 2 g each for dry mass, total fat and ash, 1 g each for sodium, magnesium, calcium and phosphorus and 0.5 g for Kjeldahl nitrogen.

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Glossary

α ..... confidence level
AAS ..... atomic absorption spectrometry
ANOVA ..... analysis of variance
AOTF-NIR ..... acousto-optical tuneable near infrared spectrometry
b ..... slope of linear regression
CRM ..... certified reference material
d_f ..... degree of freedom (regression)
DM ..... dry mass
ERM® ..... European Reference Material®
GUM ..... Guide to the Expression of Uncertainty in Measurement
ICP ..... inductively coupled plasma
IRMM ..... Institute for Reference Materials and Measurements
ISO ..... International Organization for Standardization
JRC ..... European Commission's Joint Research Centre
KFT ..... Karl Fischer titration
LOQ ..... limit of quantification
$MS_{between}$ ..... mean of squares between groups (ANOVA)
MSI ..... minimum sample intake
MS_{within} ..... mean of squares within groups (ANOVA)
n ..... number of replicates
n.c. ..... not calculable
n.d. ..... not determined
OES ..... optical emission spectrometry
p ..... level of significance
PSA ..... particle size analysis
RSD ..... relative standard deviation
RSD_{stab} ..... relative standard deviation of all results of stability study
s ..... standard deviation
s_{bb} ..... between-bottle heterogeneity standard deviation
s_{wb} ..... within-bottle heterogeneity standard deviation
se_b ..... standard error of slope b of linear regression
SI ..... International Systems of Units
u_{bb} ..... relative standard uncertainty due to between-bottle heterogeneity
u_{bb} ..... relative standard uncertainty due to heterogeneity that can be hidden by method repeatability
u_{char} ..... relative standard uncertainty of characterisation exercise
u_{CRM} ..... combined standard uncertainty of certified value
$u_{CRM, rel}$ ..... combined relative standard uncertainty of certified value
U_{CRM} ..... expanded uncertainty of certified value
$U_{CRM, rel}$ ..... expanded relative uncertainty of certified value
u_{lts} ..... relative standard uncertainty of long-term stability
u_{meas} ..... standard uncertainty of measurement result
u_{sts} ..... relative standard uncertainty of short-term stability
u_Δ ..... combined standard uncertainty of certified value and measured value
U_Δ ..... expanded uncertainty of certified value and measured value
t_{sl} ..... pre-defined shelf life
x_i ..... result at time point i in an isochronous stability study
$\overline{x}$ ..... average result of all time points in an isochronous stability study
$\overline{y}$ ..... average of all results of a homogeneity study
Δ ..... difference between two measurement results
Δ_m ..... difference between measured and certified value
$v_{MSwithin}$ ..... degrees of freedom (ANOVA)

1 Introduction

1.1 Background

This report describes the development of one reference material, which will replace the exhausted CRM BCR-384 (lyophilised pork muscle).

Knowledge of the nutritional content of foods is necessary to study the relation between diet and health, for planning of diets, in official food control, for food labelling purposes and for the manufacture of food products.

There is a growing awareness that dietary factors are important in the development of certain diseases, for example, the relationship between high fat consumption and heart disease in western societies. Many countries have therefore taken steps to improve the dietary habits of their populations, by publishing guidelines for a healthy diet. Nutritional labelling is essential for those consumers who use these guidelines to establish a balanced diet.

The food industry relies on quality control programmes involving the measurement of components in food products. Nutrition research and counselling rely heavily on analytical data for the component content of foods. This information is compiled in national food tables and component databases, frequently supported by governmental surveillance programmes to determine whether recommended dietary intakes are met within the population or segments of the European Community.

The importance of reliable consumer information in the Community is reflected in the issuing of Directive 90/496 on nutrition labelling for foodstuffs [3] amended by Regulation 1882/2003 [4] and Directive 2003/120 [5] as well as by Directive 2000/13 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs [6]. Official Food Control laboratories are charged with verification that the information provided is correct. Thus, chemical analyses of components in foods form the basis of much of the science and practice of nutrition and dietetics and is required for enforcement of Community legislation.

Similar to the exhausted CRM, it was intended to certify the mass fractions of Kjeldahl nitrogen, total fat, ash, potassium, sodium, magnesium, calcium, chlorine and phosphorus. The pork muscle reference material will provide a basis for quality control of the measurements of several nutrient components commonly measured in meat.

1.2 Expression of results

The results for the major components are expressed in g/100 g to be consistent with Directive 90/496 [3] on food labelling and food composition tables.

Throughout this report, results are expressed as a mass fraction on a dry mass basis. For practical purposes, the dry mass is established by determining the "loss of mass on drying" under carefully defined conditions (see also Sections 6.1 and 9.2). It should be noted that determination of the dry mass correction factor under conditions other than specified in this report might lead to results, which are incompatible with the certified values.

2 Participants

Project management and evaluation

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(Work performed under ISO Guide 34 accreditation; BELAC No. 268-TEST)

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(Measurements performed under ISO/IEC 17025 accreditation; DAP-PL-3295.02)

3 Processing of the material

3.1 Material selection and processing

The raw material was chosen as lean pork meat in order to minimise the fat content as this could cause handling problems in the dried material (sticky, difficult to manipulate and fill). About 300 kg of mignonette-type meat (tenderloin) were purchased from a local butcher. Visible fat, cartilage, bone and connective tissue was removed before mincing the meat in a cutter. The finely minced material was split into six sub-batches and delivered to IRMM. Approx. 60 mg/kg of α-tocopherol (ethanolic solution) was added as protecting agent (antioxidant) to each sub-batch, which was then mixed for 1 h in a stainless steel mixer (IKA-Werke GmbH & Co. KG, Staufen, DE). The meat was then spread on trays and dried in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE). The resulting cakes were manually crushed and then processed in a jaw crusher (Retsch GmbH, Haan, DE) to obtain pieces of approx. 5 mm. After cryogenic milling using a Palla vibrating mill (KHD Humboldt Wedag GmbH, Köln, DE), the powders of the sub-batches were merged and sieved (≤ 500 µm) using an industrial sieve (Russel Finex Ltd., London, GB), resulting in about 50 kg of bulk material.

The pork meat powder was homogenised using a Turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, CH). Due to a rather large sample intake, which is required to measure all certified parameters, the customer will be provided with a set of two 100 mL amber glass vials. Filling into 100 mL amber glass vials was performed in a glove box under protective atmosphere (argon) using a vibrating feeder (Laborette 24V, Fritsch GmbH, Idar-Oberstein, DE). Lyo-inserts were immediately pressed down manually into the neck of the vial thereby providing an inert atmosphere over the material. Capping and labelling took place in a capping and labelling assembly from Bausch+Ströbel Maschinenfabrik Ilshofen GmbH+Co (Ilshofen, DE) and BBK – Etikettier- und Sondermaschinenbau GmbH (Berfelden, DE), respectively. For each set two vials were put into a transparent bag. All sets were stored at -20 °C. In total, 1401 sets were produced, each comprising of two vials filled with about 18 g of lyophilised pork muscle powder.

3.2 Additional characterisation measurements

Water content of each vial of the final material was determined by online acousto-optical tuneable near infrared spectrometry (AOTF-NIR). Details can be found elsewhere [7]. Moreover, Karl Fischer titration (KFT) [8] was performed on ten vials chosen from the final material following a random stratified sample-picking scheme and analysed in duplicate. The determined mean water content of the lyophilised pork muscle material was 3.33 g/100 g (s = 0.16 g/100 g).

Particles size analysis (PSA) was performed using laser diffraction spectrometry on six vials chosen from the final material using a random stratified sample-picking scheme and analysed over a range of 0.5 to 875 µm using a Helos laser light scattering instrument (Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, DE). The determined top particle size for the lyophilised pork muscle material was 735 µm. About 50 % of all particles were smaller than 150 µm and approximately 1 % of all particles were smaller than 5 µm.

4 Homogeneity study

4.1 Design of the homogeneity study

For the homogeneity study, 48 vials (= 24 sets; ~ 1.7 % of the total batch) of ERM®-BB384 (lyophilised pork muscle) were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity, the analyses per vial were split into three groups. In the first group (16 vials) triplicate determinations of total fat content were performed. In the second group (16 vials) triplicate determinations of Kjeldahl nitrogen and chlorine content were performed and in the third group (16 vials) triplicate determinations of ash, sodium, potassium, magnesium, calcium and phosphorus content were performed. Details for the analytical methods used are given in Tables 4 and 5 (see Section 6.1; lab code 8). As the contents of potassium and chlorine could not be certified due to technical reasons (see Section 6.2), no results from the homogeneity study are reported here.

Samples were measured in a random order (predefined at IRMM and communicated to the laboratory) to allow distinction between an analytical trend and a trend in the filling sequence. As all required measurements per measurand could not be performed within one day, they were split over three days (for each of the vials one replicate measurement per day). In order to exclude the influence of the day-to-day variance, two-way analysis of variance (ANOVA) was applied. In each of the 24 sets duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination. Individual results can be seen in the Annex (Tables A1 to A7).

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying individual results as well as outlying bottle averages. Regression analyses were performed to detect possible trends regarding analytical or filling sequence. The uncertainty contribution from possible heterogeneity was estimated by ANOVA [9]. Method repeatability (s_{wb}) expressed as a relative standard deviation is given in equation 1:

$$s_{wb} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad (1)$$

MS_{within} = mean square within a bottle from an ANOVA

$\bar{y}$ = average of all results of a homogeneity study

Between-unit variability (s_{bb}) expressed as a relative standard deviation is given by equation 2:

$$s_{bb} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad (2)$$

$MS_{between}$ = mean square among bottles from an ANOVA

n = average number of replicates per bottle

Heterogeneity that can be hidden by method repeatability is defined in equation 3:

$$u_{bb}^{*} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (3)$$

$\nu_{MSwithin}$ = degrees of freedom of MS_{within}

The larger value of s_{bb} or u^{*}_{bb} was used as uncertainty contribution for heterogeneity, u_{bb} (see Table 1 for a summary of results, values were converted into relative uncertainties).

4.2 Results of the homogeneity study

No outliers were detected. No significant slopes were found for analytical nor for filling sequences. In conclusion, the distribution of all seven proximates and essential elements in this material can be considered as homogeneous.

Table 1: Evaluation of homogeneity study for proximates and essential elements in ERM®-BB384 (lyophilised pork muscle)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**
Mean [g/100 g]	13.99	9.03	4.58
RSD [%]	2.93	1.72	2.98
MS_{within}	0.09226	0.01747	0.00322
$MS_{between}$	0.14859	0.02659	0.00284
s_{wb} [%]	2.17	1.46	1.24
s_{bb} [%]	0.98	0.61	n.c. [1)]
u^*_{bb} [%]	0.64	0.43	0.36
u_{bb} [%]	**0.98**	**0.61**	**0.36**

Essential elements	**Na**	**Mg**	**Ca**	**P**
Mean [mg/g]	1.88	1.08	0.17	8.96
RSD [%]	3.17	2.59	2.99	2.21
MS_{within}	0.00152	0.00062	0.00001	0.01230
$MS_{between}$	0.00760	0.00087	0.00006	0.09750
s_{wb} [%]	2.08	2.29	1.70	1.24
s_{bb} [%]	2.40	0.84	2.51	1.88
u^*_{bb} [%]	0.61	0.67	0.50	0.36
u_{bb} [%]	**2.40**	**0.84**	**2.51**	**1.88**

1) n.c. = not calculable because $MS_{between} < MS_{within}$

4.3 Minimum sample intake

The minimum sample intakes for the lyophilised pork muscle material were established based on the sample intakes used for the measurements for the homogeneity and stability studies as well as for the characterisation (if accepted data sets were submitted). For details, see also Tables 4 and 5 in Section 6.1.

The minimum sample intakes are 2 g each for dry mass, total fat and ash, 1 g each for sodium, magnesium, calcium and phosphorus and 0.5 g for Kjeldahl nitrogen.

5 Stability studies

5.1 Short-term stability study

5.1.1 Design of the short-term stability study

A four weeks isochronous study [10] was performed to evaluate stability of the lyophilised pork muscle material during transport. For the short-term stability study, 28 vials (= 14 sets) were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity, the 28 vials were split into two groups. In the first group (14 vials) duplicate determinations of dry mass, Kjeldahl nitrogen and total fat content were performed. In the second group (14 vials) duplicate determinations of dry mass, ash, potassium, sodium, magnesium, calcium, chlorine and phosphorus content were performed. Details for the analytical methods used are given in Tables 4 and 5 (see Section 6.1; lab code 8). As the contents of potassium and chlorine could not be certified due to technical reasons (see Section 6.2), no results from the short-term stability study are reported here.

Samples were stored at 18 °C and 60 °C as well as at a reference temperature of -20 °C. Two vials were stored at each temperature for 0, 1, 2 and 4 weeks. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under intermediate precision conditions in the order predefined at IRMM (randomised sample order) using the same methods as for the homogeneity study. In order to exclude the influence of the day-to-day variance the results were normalised to the mean of the results on the particular day versus the mean of all results. In each of the 28 vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination.

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to shipping conditions (see Table 2 for a summary). The observed slopes were tested for significance using a *t*-test, with $t_{\alpha,df}$ being the critical *t*-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence level). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Graphs can be found in Annex B.

5.1.2 Results of the short-term stability study

No outliers were detected. No statistically significant slopes were detected at 99 % and 95 % confidence levels. In general, it was concluded that the uncertainties of the short-term stability (u_{sts}) can be assumed to be negligible, if sample shipment is carried out at ambient temperature, which therefore shall be the dispatch condition for sample shipment to the customer.

Table 2: Evaluation of the short-term stability study for proximates and essential elements in ERM®-BB384 (lyophilised pork muscle)

Proximates	Kjeldahl N		Total fat		Ash	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	0.84	0.05	0.37	1.77	0.04	0.59
Outlier (99 % confidence level)	none	none	none	none	none	none
Statistical significance of the slope (95 % confidence level) [1)]	no	no	no	no	no	no
Statistical significance of the slope (99 % confidence level) [2)]	no	no	no	no	no	no
u_{sts} [%/week]	0.38	0.39	0.31	0.22	0.26	0.28

Essential elements	Na		Mg		Ca		P	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	0.65	1.38	0.81	0.78	1.41	0.15	1.00	1.74
Outlier (99 % confidence level)	none	none	none	none	none	none	none	none
Statistical significance of the slope (95 % confidence level) [1)]	no	no	no	no	no	no	no	no
Statistical significance of the slope (99 % confidence level) [2)]	no	no	no	no	no	no	no	no
u_{sts} [%/week]	0.35	0.35	0.23	0.19	0.33	0.37	0.17	0.16

1) $t_{0.05;14} = 2.145$
2) $t_{0.01;14} = 2.977$

5.2 Long-term stability study

5.2.1 Design of the long-term stability study

A 24 months isochronous study [9] was performed to evaluate stability of the lyophilised pork muscle material during storage. For the long-term stability study, 24 vials (= 12 sets) were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity, the 24 vials were split into two groups. In the first group (12 vials) duplicate determinations of dry mass, Kjeldahl nitrogen and total fat content were performed. In the second group (12 vials) duplicate determinations of dry mass, ash, potassium, sodium, magnesium, calcium, chlorine and phosphorus content were performed. Details for the analytical methods used are given in Tables 4 and 5 (see Section 6.1; lab code 7). As the contents of potassium and chlorine could not be certified due to technical reasons (see Section 6.2), no results from the short-term stability study are reported here.

Samples were stored at 4 °C as well as at a reference temperature of -20 °C. Three vials were stored at each temperature for 0, 8, 16 and 24 months. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under intermediate precision conditions in the order predefined at IRMM (randomised sample order). In order to exclude the influence of the day-to-day variance the results were normalised to the mean of the results on the particular day versus the mean of all results. In each of the 24 vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination.

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to storage conditions (see Table 3 for a

summary). The observed slopes b were tested for significance using a t-test, with $t_{\alpha,df}$ being the critical t-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence level). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Finally, the uncertainty of stability u_{lts} [11] was calculated for a pre-defined shelf life of 24 months applying equation 4:

$$u_{lts} = \frac{RSD_{stab}}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl} \qquad (4)$$

with RSD_{stab} being the relative standard deviation of all 48 individual results of the relevant stability study, x_i being the time point for each replicate, $\bar{x}$ being the average of all time points and t_{sl} being the pre-defined shelf-life. Graphs can be found in Annex C.

5.2.2 Results of the long-term stability study

No outliers were detected. No statistically significant slopes were detected at 99 % confidence level. A statistically significant positive slope (95 % confidence level) was detected for Kjeldahl nitrogen content. Although it is rather unlikely that the nitrogen content is increasing during storage, potential degradation was included into the calculation of u_{lts} (without degradation u_{lts} would result in 0.27 %/24 months). A statistically significant negative slope (95 % confidence level) was detected for total fat content, which is mainly driven by a single result of the 24 months data. Removing it would result in a non-significant slope. Moreover, the slope might be emphasised because of the good method repeatability. Nevertheless, all data were considered in the calculation and consequently, potential degradation was included into the calculation of u_{lts} (without degradation u_{lts} would result in 0.21 %/24 months). The stability of the material under these conditions was demonstrated, but further stability monitoring will especially focus on Kjeldahl nitrogen and total fat content. 4 °C was chosen as storage temperature for the whole batch.

Table 3: Evaluation of the 24 months long-term stability study for proximates and essential elements in ERM®-BB384 (lyophilised pork muscle)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**
$\|b\|/se_b$	2.67	2.21	0.47
Outlier (99 % confidence level)	none	none	none
Statistical significance of the slope (95 % confidence level) [1)]	yes	yes	no
Statistical significance of the slope (99 % confidence level) [2)]	no	no	no
u_{lts} [%/24 months]	**0.50**	**0.33**	**1.29**

Essential elements	**Na**	**Mg**	**Ca**	**P**
$\|b\|/se_b$	0.48	0.46	0.42	0.53
Outlier (99 % confidence level)	none	none	none	none
Statistical significance of the slope (95 % confidence level) [1)]	no	no	no	no
Statistical significance of the slope (99 % confidence level) [2)]	no	no	no	no
u_{lts} [%/24 months]	**0.79**	**0.85**	**2.71**	**0.75**

1) $t_{0.05;22} = 2.074$
2) $t_{0.01;22} = 2.819$

6 Characterisation

6.1 Design of the characterisation study

The certification exercise was performed in 2007. Eleven laboratories were carefully selected to perform the analytical measurements. Validated methods were an indispensable requirement for participation; an accredited method was considered an asset. The laboratories had to prove their measurement capabilities and had to demonstrate previous experience in the analysis of proximates and essential elements in comparable matrices.

Each laboratory was provided with six vials of ERM®-BB384 (lyophilised pork muscle). Because of the limited sample quantity, the analyses per vial were split into two groups. In the first group (three vials) duplicate determinations of Kjeldahl nitrogen and total fat content were performed. In the second group (three vials) duplicate determinations of ash, potassium, sodium, magnesium, calcium, chlorine and phosphorus content were performed. The measurements per analyte were spread over two days. In each of the six vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination. Details for the minimum sample intakes and the analytical methods used are given in Tables 4 and 5.

Table 4: Minimum sample intakes (MSI) in gram and methods used for determination of proximate contents in ERM®-BB384 (lyophilised pork muscle)

Lab code	DM [1]	Kjeldahl N	Total fat [2]	Ash [3]
	MSI	MSI	MSI	MSI
1	1.5	1.0	2.5	2.0
2	0.5	1.0	0.6	0.5
3	1.0	0.2	1.3	1.5
4	2.0	0.2	6.0	3.0
5	2.0	0.15	2.0	1.0
6	2.0	0.4	3.0	2.0
7	2.0	0.5	2.0	2.0
8	4.0 [4]	1.0	4.0	4.0
9	3.0	1.0	3.0	3.0
10	2.0	0.5	5.0	2.5
11	2.0	0.8 [5]	2.0	3.0

1) Dry mass determination to be performed at 100 – 102 °C for 16 – 18 h according to procedure AOAC 950.46
2) Total fat determination (gravimetric) to be performed after acid hydrolysis and solvent extraction [12]
3) Ash determination to be performed at 550 °C ± 25 °C
4) Dry mass determination was performed at 102 °C (3 h)
5) Nitrogen content was determined by Dumas method

The following variations of the Kjeldahl nitrogen determination were noticed: Different digestors (Büchi, Foss, Gebhardt) were used. Digestion was performed using sulphuric acid with or without H_2O_2 and different catalyst combinations ($CuS0_4$ + TiO_2, Se + K_2SO_4, $CuSO_4$ + K_2SO_4) with varying digestion times (1 to 3 h).

The following variations of the total fat determination were noticed: Hydrolysis was performed using different concentrations of hydrochloric acid (10 to 50 %) with varying hydrolysis times (1 to 6 h). Extraction was performed using petrol ether or a mixture of petrol ether with diethyl ether with varying extraction times (1 to 8 h).

Table 5: Minimum sample intakes (MSI) in g and methods used for determination of essential element contents in ERM®-BB384 (lyophilised pork muscle)

Lab code	K		Na		Mg		Ca		Cl		P	
	MSI	Method	MSI	Method	MSI	Method	MSI	Method	MSI	Method	MSI	Method
1	2.0	flame OES	2.0	flame OES	2.0	flame AAS	2.0	flame AAS	1.5	titrimetry	2.0	spectro-photometry
2	0.2	ICP-OES	0.2	ICP-OES	0.2	ICP-OES	0.2	ICP-OES	0.2	ICP-OES	0.2	ICP-OES
3	1.8	flame photometry	1.8	flame photometry	1.5	flame AAS	1.5	flame AAS	0.5	ion chro-matography	1.8	spectro-photometry
4	0.1	flame AAS	0.1	flame AAS	0.1	flame AAS	0.1	flame AAS	4.0	potentio-metry	n.d.	n.d.
5	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	2.5	potentio-metry	1.0	ICP-OES
6	2.0	flame AAS	2.0	flame AAS	2.0	flame AAS	2.0	flame AAS	2.0	potentio-metry	2.0	spectro-photometry
7	2.0	ICP-OES	2.0	ICP-OES	2.0	ICP-OES	2.0	ICP-OES	2.0	ICP-OES	2.0	ICP-OES
8	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	titrimetry	1.0	ICP-OES
9	5.0	flame photometry	5.0	flame photometry	5.0	flame AAS	3.0	gravimetry	1.0	potentio-metry	1.0	spectro-photometry
10	0.2	flame OES	0.2	flame OES	0.2	flame AAS	0.2	flame AAS	2.0	potentio-metry	0.2	spectro-photometry
11	3.0	ICP-OES	3.0	flame photometry	3.0	ICP-OES	3.0	ICP-OES	3.0	potentio-metry	3.0	ICP-OES

n.d. = not determined

6.2 Results and technical evaluation

After receipt of the data sets, the results were subjected to technical evaluation. The accepted sets of results were submitted to the following statistical tests:

- Scheffe multiple *t*-test to check if the means of two labs are significantly different
- Dixon test to detect outlying laboratory means
- Grubbs test to detect single and double outliers
- Cochran test to check for outlying laboratory variances
- Bartlett test to check for homogeneity of laboratory variances
- ANOVA to assess between laboratory and within laboratory variances and test their significance employing the Snedecor *F*-test
- Skewness and kurtosis tests to assess the normality of the lab means distribution

The results of the statistical tests of the finally considered data for ERM®-BB384 (lyophilised pork muscle) are summarised in Table 6. Individual results and corresponding graphs can be found in Annex D.

All data sets from Lab 2 were excluded as the sample intake for dry mass determination was much too low, thus were leading to erroneous results.

Kjeldahl N Lab 11 submitted a data set obtained by the Dumas method. Although the results fit very well into the data sets of the other participants, only data sets obtained by the Kjeldahl method were kept as this was previously requested. Therefore, nine data sets were taken into account for certification.

Total fat One outlying data set was detected by the Grubbs test. As there were no technical reasons for exclusion, the data set was checked according to ERM Application Note 1 [13]. No significant difference was found, thus, the data set was kept. In total, ten data sets were taken into account for certification.

Ash Data set from Lab 10 was excluded because it did not meet the specifications of the lab (repeatability and reproducibility). Therefore, nine data sets were taken into account for certification.

Sodium Ten data sets were taken into account for certification.

Potassium Because of the heterogeneity of the data sets, the potassium content was not taken into account for certification.

Magnesium Data set from Lab 4 was excluded because it did not meet the specifications of the lab (repeatability and reproducibility). In total, nine data sets were taken into account for certification.

Calcium Lab 6 withdrew its data set because of technical problems. Lab 9, the only lab using a gravimetric method, confirmed that the provided sample quantity was not sufficient for this method. Therefore, this data set was excluded for technical reasons. Therefore, eight data sets were taken into account for certification.

Chlorine Labs 5 and 6 reported that the content was below the labs' LOQ, therefore no data sets were submitted. Because of the heterogeneity of the remaining data sets, the chlorine content was not taken into account for certification.

Phosphorus Lab 4 did not measure this analyte. Therefore, nine data sets were taken into account for certification.

In all cases, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Moreover, it was decided to keep those data sets, which have outlying laboratory variances (Cochran test) as not technical reasons could be given.

Table 6: Summary of statistical evaluation for proximates and essential elements in ERM®-BB384 (lyophilised pork muscle)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**
Number of data sets	9	10	9
Number of replicate measurements	54	60	54
Mean of means [g/100 g]	14.17	8.99	4.51
Relative standard deviation of mean of means [%]	1.74	2.55	4.50
Relative standard error of mean of means (u_{char}) [%]	0.58	0.81	1.50
All data sets compatible two by two? (Scheffe test)	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	yes (Lab 11)	none
Outlying lab variances? (Cochran test; p = 0.05)	yes (*Lab 5, Lab 6, Lab 10)	yes (*Lab 11)	yes (Lab 7, Lab 9)
Lab variances homogeneous? (Bartlett test; p = 0.01)	no	no	no
Variances between labs sign. different? (Snedecor F-test; p = 0.01)	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes

Essential elements	**Na**	**Mg**	**Ca**	**P**
Number of data sets	10	9	8	9
Number of replicate measurements	60	54	48	54
Mean of means [g/100 g]	1.86	1.03	0.16	8.71
Relative standard deviation of mean of means [%]	9.98	3.09	14.67	3.81
Relative standard error of mean of means (u_{char}) [%]	3.16	1.03	5.19	1.27
All data sets compatible two by two? (Scheffe test)	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	none	yes (Lab 1, Lab 3)	yes (*Lab 8, Lab 10)	none
Lab variances homogeneous? (Bartlett test; p = 0.01)	no	no	no	no
Variances between labs sign. different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes

* p = 0.01

7 Certified values and uncertainties

The certified values for ERM®-BB384 (lyophilised pork muscle) are calculated as the mean of means of the accepted data sets. The standard error of the mean of means was used as an estimation of the uncertainty contribution of the characterisation exercise. The standard error is calculated as the standard deviation divided by the square root of the number of accepted data sets.

The combined standard uncertainty of the certified value includes contributions from the between-bottle heterogeneity, long-term storage and the characterisation study. The relative combined standard uncertainty is calculated according to equation 5:

$$u_{CRM} = \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2} \qquad (5)$$

Table 7 summarises the individual uncertainty contributions and the resulting expanded uncertainties as well as the certified values and their uncertainties after rounding for ERM®-BB384 (lyophilised pork muscle).

Table 7: Certified values and uncertainties for ERM®-BB384 (lyophilised pork muscle)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**
u_{bb} [%]	0.98	0.61	0.36
u_{lts} [%] [1)]	0.50	0.33	1.29
u_{char} [%]	0.58	0.81	1.50
$u_{CRM, rel}$ [%]	1.24	1.08	2.01
$U_{CRM, rel}$ (k = 2) [%]	2.48	2.13	4.02
Certified value [g/100 g]	**14.2**	**8.99**	**4.51**
U_{CRM} (k = 2) [g/100 g]	**0.4**	**0.20**	**0.19**

Essential elements	**Na**	**Mg**	**Ca**	**P**
u_{bb} [%]	2.40	0.84	2.51	1.88
u_{lts} [%] [1)]	0.79	0.85	2.71	0.75
u_{char} [%]	3.16	1.03	5.19	1.27
$u_{CRM, rel}$ [%]	4.04	1.58	6.37	2.39
$U_{CRM, rel}$ (k = 2) [%]	8.08	3.16	12.73	4.78
Certified value [mg/g]	**1.86**	**1.03**	**0.164**	**8.7**
U_{CRM} (k = 2) [mg/g]	**0.15**	**0.04**	**0.021**	**0.5**

1) Shelf life 24 months

8 Metrological traceability

The measurement results for assigning nitrogen mass fraction values are method dependent (Kjeldahl). They were obtained by different digestion procedures and subsequent quantification by Kjeldahl methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning total fat mass fraction values are method dependent. They are obtained by different procedures for acid hydrolysis and solvent extraction and subsequent quantification by gravimetric methods. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning ash mass fraction values are method dependent. They are obtained by gravimetric methods based on ashing at 550 °C ± 25 °C. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning sodium, magnesium and calcium mass fraction values are obtained by different digestion and extraction procedures and subsequent quantification by ICP-OES, flame OES, flame AAS and flame photometric methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning phosphorus mass fraction values are obtained by different digestion and extraction procedures and subsequent quantification by ICP-OES and spectrophotometric methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

9 Instructions for use and intended use

9.1 Safety precautions

The usual laboratory safety precautions apply.

9.2 Use of materials

- Allow the vial to warm up to ambient temperature before opening.
- Shake vial before aliquotation.
- Certified values are based on dry mass.
- Dry mass determination should be performed at least in duplicate.
- To determine dry mass weigh accurately an aliquot of approximately 2 g on an analytical balance. The weighing should be performed immediately after opening of the vial to minimise potential water uptake or release by the lyophilised pork muscle material. Drying has to be performed at 100 - 102 °C for 16 - 18 h (according to procedure AOAC 950.46).

9.3 Intended use

This material is intended to be used for method performance control and validation purposes. For assessing the method performance, the measured values of the CRMs are compared with the certified values following a procedure described by Linsinger [13]. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}) according to equation 6:

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad (6)$$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two ($k = 2$), corresponding to a confidence level of approximately 95 %.
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value at a confidence level of about 95 %.

9.4 Storage conditions

The materials should be stored at a temperature of 4 °C ± 3 °C. However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially after opening of the vials.

10 Acknowledgements

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Annex A ERM®-BB384 (lyophilised pork muscle) – Results of the homogeneity study

Table A1: Data of homogeneity study measurements of Kjeldahl nitrogen content in ERM®-BB384 (related to dry mass)

Kjeldahl nitrogen mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-2	14.35	14.10	13.39
134-2	14.11	14.47	13.69
285-1	14.24	13.61	13.37
451-1	14.34	13.56	13.61
500-2	14.34	13.47	13.51
624-2	14.37	13.52	13.58
902-1	14.43	14.34	14.51
972-2	14.41	13.85	14.52
1011-1	14.43	14.37	13.32
1046-1	14.32	14.31	13.39
1046-2	14.57	14.35	14.50
1126-1	14.09	14.31	13.63
1194-2	14.31	13.47	13.80
1234-1	14.32	13.62	13.74
1234-2	14.26	13.60	13.64
1320-1	14.34	13.54	13.66

Table A2: Data of homogeneity study measurements of total fat content in ERM®-BB384 (related to dry mass)

Total fat mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
88-1	9.04	9.13	9.06
139-1	8.91	9.09	9.08
213-1	8.94	8.89	9.10
342-2	8.53	9.01	9.08
424-1	9.15	9.21	9.10
500-1	9.10	8.91	9.09
624-1	8.77	9.00	8.88
704-1	8.85	8.87	9.16
741-2	9.06	9.11	9.19
803-2	8.93	9.23	9.21
870-2	8.96	9.11	9.11
902-2	8.97	8.68	9.20
1011-2	9.13	9.24	9.24
1126-2	9.05	8.98	8.95
1194-1	9.03	9.08	9.06
1355-2	8.63	9.13	9.08

Table A3: Data of homogeneity study measurements of ash content in ERM®-BB384 (related to dry mass)

Ash mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-1	4.64	4.42	4.56
88-2	4.69	4.40	4.68
134-1	4.73	4.43	4.75
139-2	4.73	4.31	4.70
213-2	4.70	4.35	4.68
285-2	4.65	4.53	4.58
342-1	4.69	4.43	4.73
424-2	4.55	4.44	4.56
451-2	4.68	4.49	4.57
704-2	4.59	4.38	4.64
741-1	4.70	4.44	4.69
803-1	4.67	4.37	4.69
870-1	4.67	4.41	4.70
972-1	4.68	4.37	4.67
1320-2	4.62	4.33	4.70
1355-1	4.70	4.37	4.68

Table A4: Data of homogeneity study measurements of sodium content in ERM®-BB384 (related to dry mass)

Sodium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-1	1.89	1.97	1.91
88-2	1.88	1.94	1.93
134-1	1.95	1.95	1.94
139-2	1.91	1.85	1.90
213-2	1.74	1.83	1.81
285-2	1.77	1.85	1.84
342-1	1.85	1.85	1.83
424-2	1.70	1.80	1.83
451-2	1.87	1.87	1.90
704-2	1.88	1.87	1.93
741-1	1.86	1.87	1.87
803-1	1.99	1.90	1.96
870-1	1.96	1.90	1.87
972-1	1.88	1.92	1.87
1320-2	1.84	1.86	1.96
1355-1	1.84	1.83	1.92

Table A5: Data of homogeneity study measurements of magnesium content in ERM®-BB384 (related to dry mass)

Magnesium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-1	1.12	1.10	1.11
88-2	1.07	1.07	1.06
134-1	1.06	1.07	1.10
139-2	1.06	1.06	1.07
213-2	1.03	1.09	1.05
285-2	1.05	1.07	1.06
342-1	1.04	1.15	1.07
424-2	1.05	1.07	1.10
451-2	1.05	1.12	1.08
704-2	1.06	1.08	1.04
741-1	1.10	1.06	1.11
803-1	1.11	1.11	1.06
870-1	1.05	1.09	1.11
972-1	1.06	1.11	1.08
1320-2	1.07	1.13	1.11
1355-1	1.08	1.09	1.14

Table A6: Data of homogeneity study measurements of calciumcontent in ERM®-BB384 (related to dry mass)

Calcium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-1	0.17	0.17	0.17
88-2	0.17	0.17	0.16
134-1	0.18	0.18	0.17
139-2	0.17	0.17	0.18
213-2	0.17	0.17	0.17
285-2	0.17	0.17	0.17
342-1	0.18	0.17	0.18
424-2	0.17	0.17	0.17
451-2	0.17	0.17	0.17
704-2	0.16	0.17	0.16
741-1	0.17	0.18	0.18
803-1	0.18	0.17	0.17
870-1	0.18	0.17	0.18
972-1	0.17	0.17	0.17
1320-2	0.18	0.17	0.17
1355-1	0.18	0.18	0.18

Table A7: Data of homogeneity study measurements of phosphorus content in ERM®-BB384 (related to dry mass)

Phosphorus mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
43-1	9.25	9.11	9.23
88-2	8.91	8.93	8.80
134-1	8.95	9.08	9.21
139-2	9.01	8.94	8.85
213-2	8.50	8.62	8.53
285-2	8.81	9.04	8.90
342-1	8.75	8.75	8.73
424-2	8.71	8.71	8.64
451-2	9.08	9.16	9.12
704-2	9.06	8.97	8.88
741-1	9.20	9.19	9.05
803-1	8.73	8.82	9.15
870-1	9.08	8.97	9.11
972-1	9.00	8.97	9.12
1320-2	8.85	9.05	9.27
1355-1	9.28	9.10	9.02

Annex B ERM®-BB384 (lyophilised pork muscle) – Results of the short-term stability study

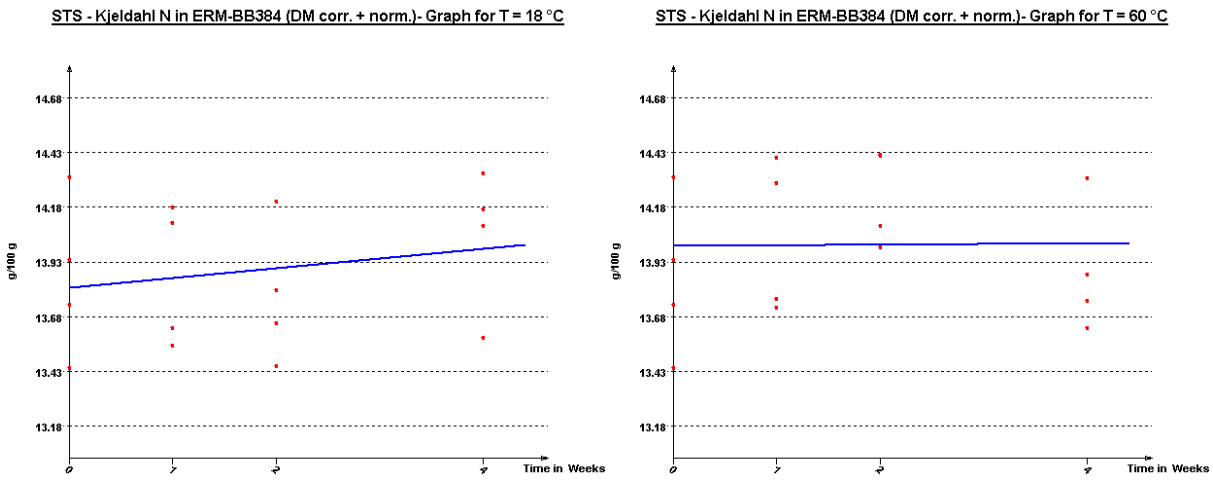


Figure B1: Short-term stability of Kjeldahl nitrogen content in ERM®-BB384 at 18 and 60 °C.

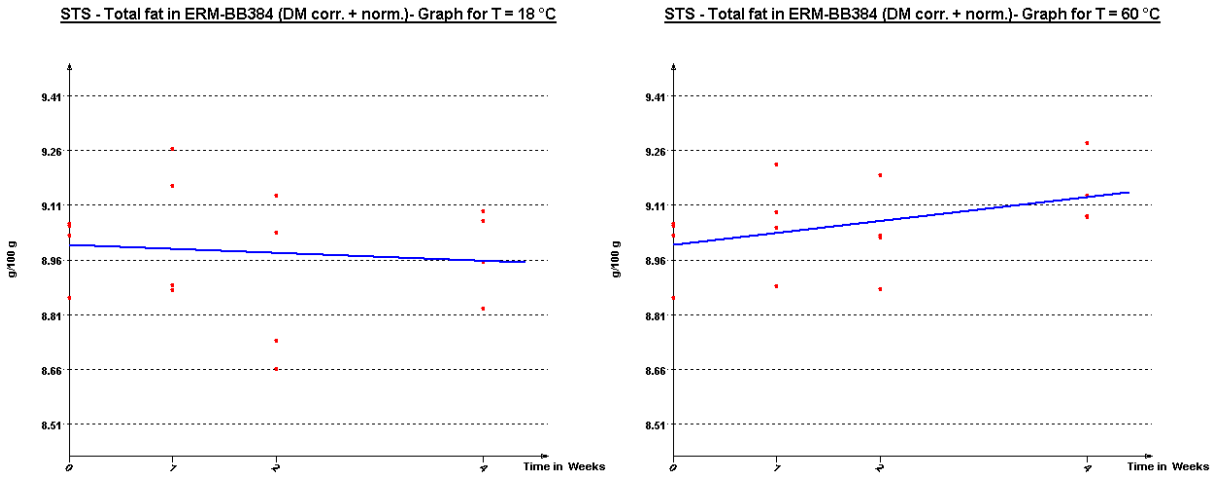


Figure B2: Short-term stability of total fat content in ERM®-BB384 at 18 and 60 °C.

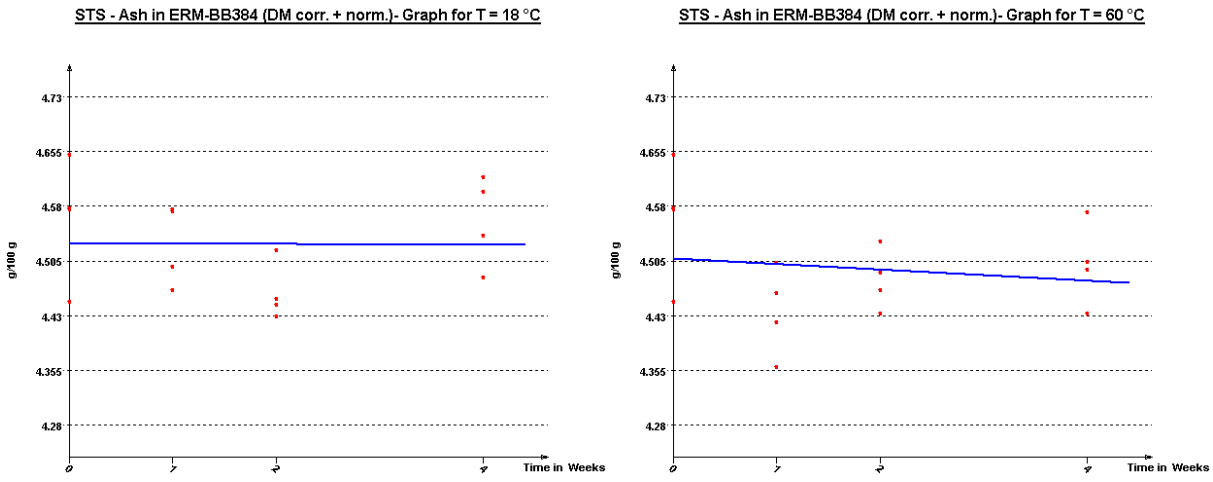


Figure B3: Short-term stability of ash content in ERM®-BB384 at 18 and 60 °C.

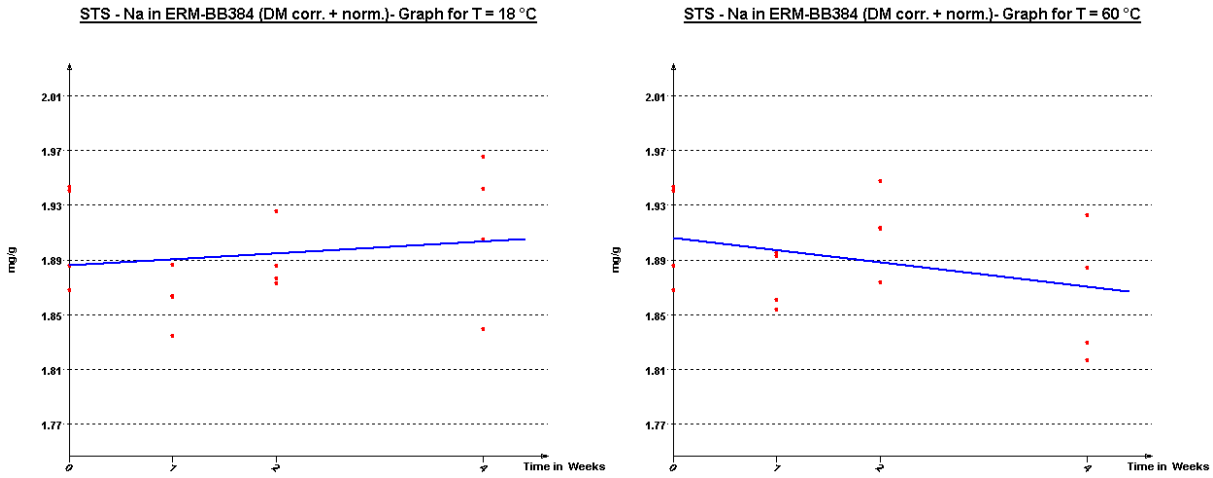


Figure B4: Short-term stability of sodium content in ERM®-BB384 at 18 and 60 °C.

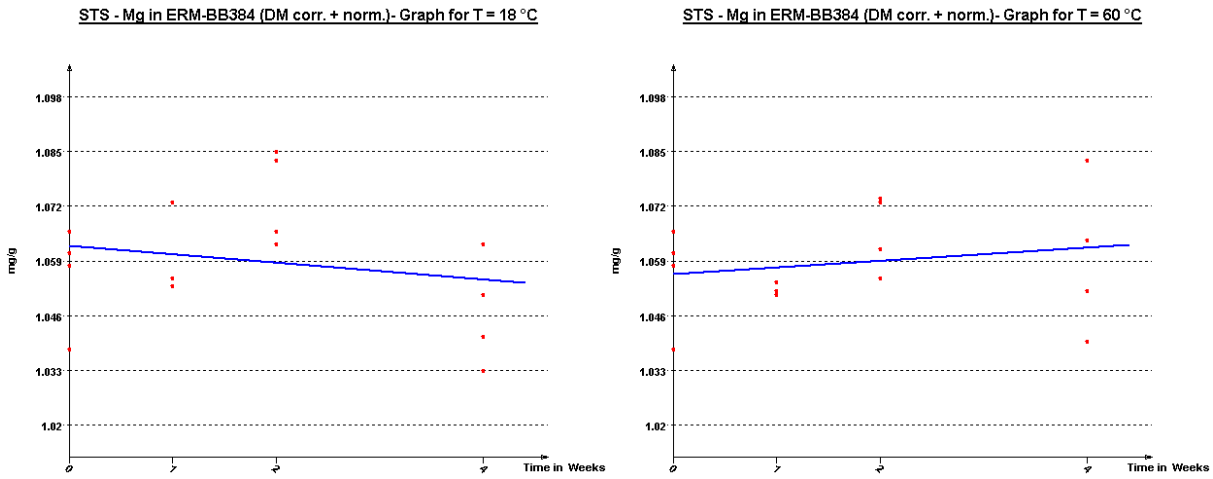


Figure B5: Short-term stability of magnesium content in ERM®-BB384 at 18 and 60 °C.

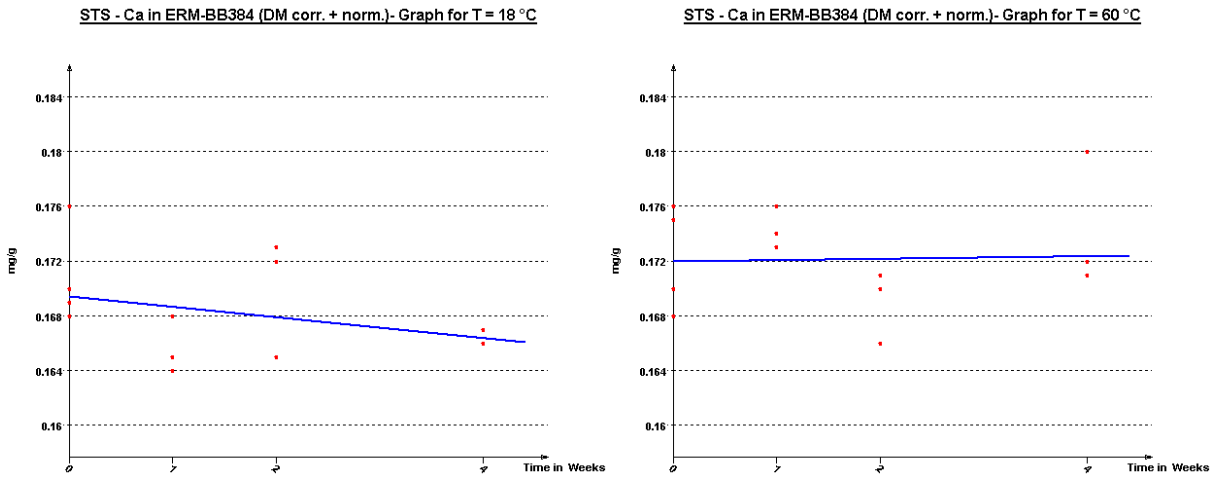


Figure B6: Short-term stability of calcium content in ERM®-BB384 at 18 and 60 °C.

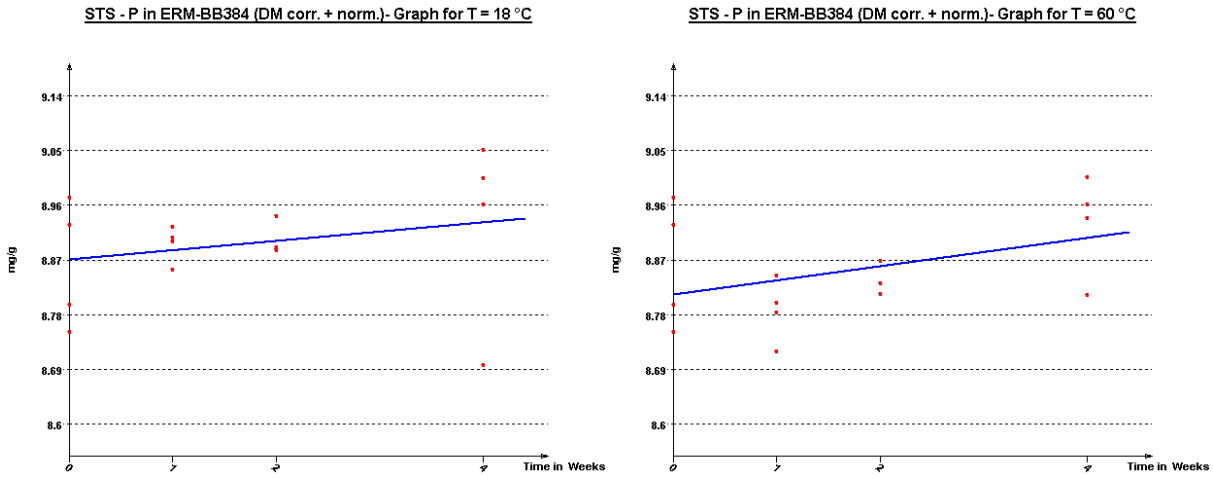


Figure B7: Short-term stability of phosphorus content in ERM®-BB384 at 18 and 60 °C.

Annex C ERM®-BB384 (lyophilised pork muscle) – Results of the long-term stability study

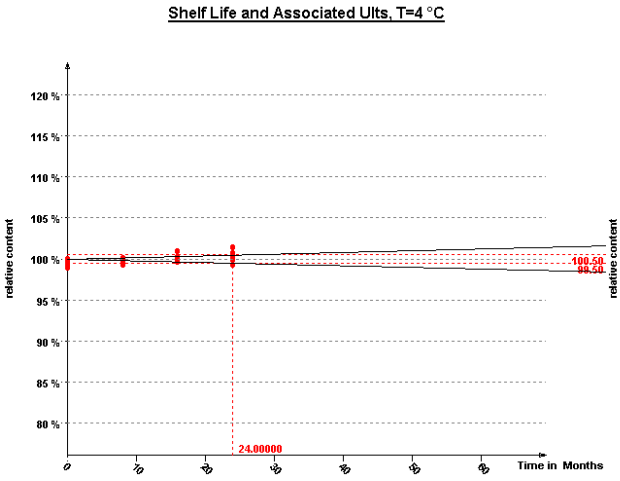
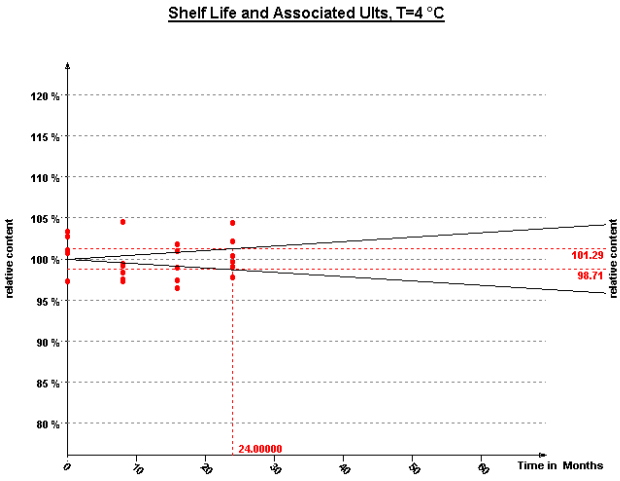


Figure C1 (left): Long-term stability of Kjeldahl nitrogen content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C2 (right): Long-term stability of total fat content in of ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.



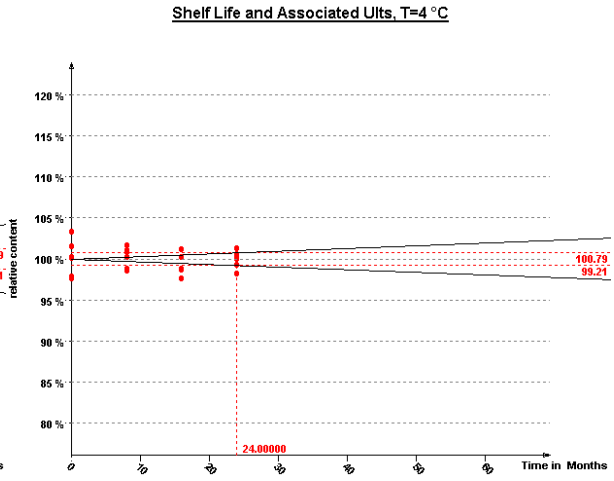
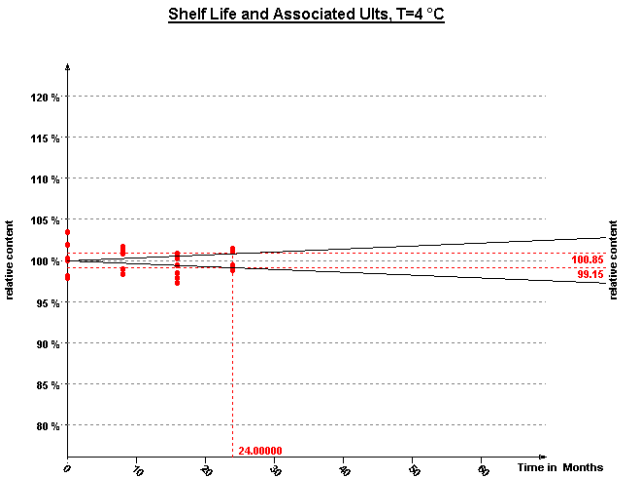


Figure C3 (left): Long-term stability of ash content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C4 (right): Long-term stability of sodium content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.



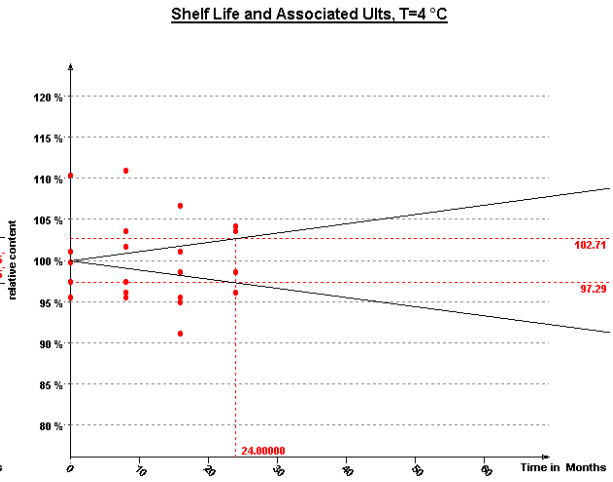
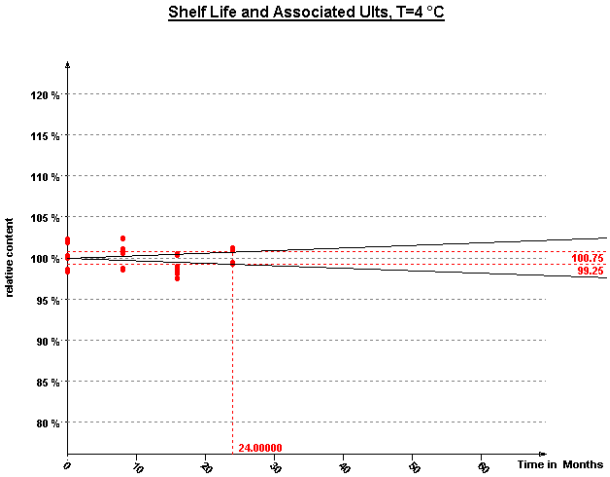


Figure C5 (left): Long-term stability of magnesium content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C6 (right): Long-term stability of calcium content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.



Figures C7: Long-term stability of phosphorus content in ERM®-BB384 at 4 °C with associated u_{lts} for storage period of 24 months.

Annex D ERM®-BB384 (lyophilised pork muscle) – Characterisation data

Table D1: Results of characterisation measurements of Kjeldahl nitrogen content in ERM®-BB384 (related to dry mass)

	Kjeldahl nitrogen mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]					
Lab code	**Day 1**			**Day 2**		
1	13.71	13.72	13.76	13.65	13.75	13.88
3	14.01	13.97	13.96	13.96	13.93	13.97
4	14.32	14.18	14.20	14.39	14.20	14.24
5	14.23	14.35	15.24	14.40	14.44	14.39
6	14.46	14.68	14.74	14.38	14.41	14.38
7	14.31	14.25	14.30	14.11	14.07	14.12
8	14.12	14.21	14.16	14.29	14.21	14.25
9	14.03	13.96	14.09	14.11	14.01	14.03
10	13.79	13.96	14.13	14.08	14.25	14.33

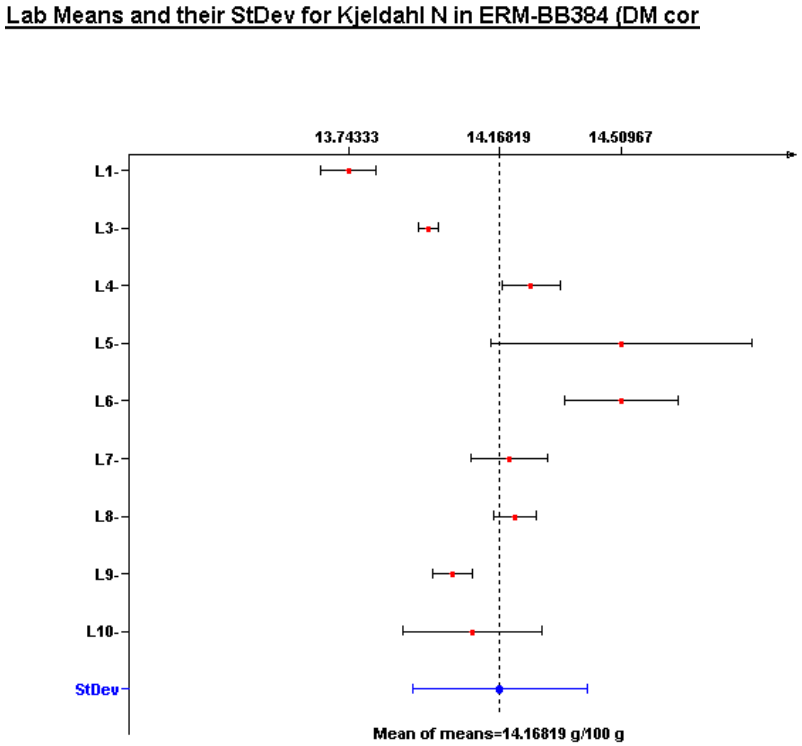


Figure D1: Laboratory means, mean of means and corresponding standard deviations for Kjeldahl nitrogen in ERM®-BB384 (related to dry mass)

Table D2: Results of characterisation measurements of total fat content in ERM®-BB384 (related to dry mass)

	Total fat mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]					
Lab code	**Day 1**			**Day 2**		
1	9.36	9.18	9.40	9.37	9.23	9.10
3	9.08	9.05	9.01	9.13	8.99	9.01
4	9.14	9.23	9.22	9.14	9.15	9.18
5	8.95	9.06	9.16	8.96	9.03	9.06
6	8.78	8.75	8.86	8.54	8.88	8.77
7	8.97	9.07	9.03	9.07	8.93	8.90
8	9.18	9.22	9.10	9.24	9.28	9.02
9	9.03	8.99	8.94	8.93	8.81	8.86
10	8.97	8.95	9.04	9.07	9.08	9.03
11	8.21	8.43	8.54	8.32	8.53	8.85

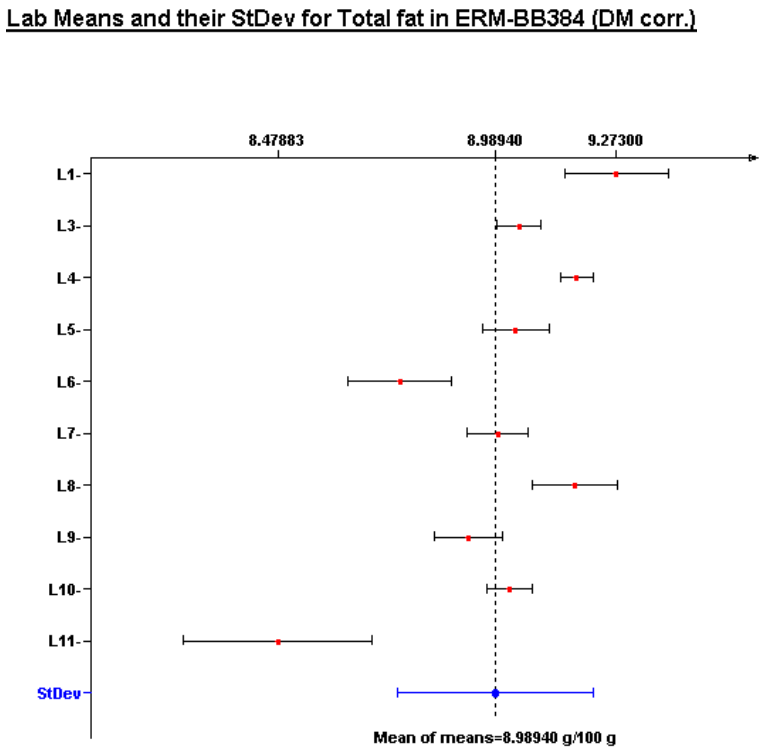


Figure D2: Laboratory means, mean of means and corresponding standard deviations for total fat in ERM®-BB384 (related to dry mass)

Table D3: Results of characterisation measurements of ash content in ERM®-BB384 (related to dry mass)

	Ash mass fraction in ERM®-BB384 (lyophilised pork muscle) [g/100 g]					
Lab code	**Day 1**			**Day 2**		
1	4.33	4.37	4.31	4.41	4.51	4.42
3	4.84	4.82	4.82	4.78	4.78	4.79
4	4.45	4.45	4.46	4.46	4.45	4.47
5	4.59	4.69	4.59	4.70	4.67	4.66
6	4.55	4.53	4.51	4.50	4.48	4.50
7	4.67	4.66	4.88	4.67	4.81	4.88
8	4.34	4.32	4.49	4.31	4.42	4.31
9	4.00	4.06	4.38	4.09	4.16	4.27
11	4.48	4.40	4.39	4.58	4.48	4.54

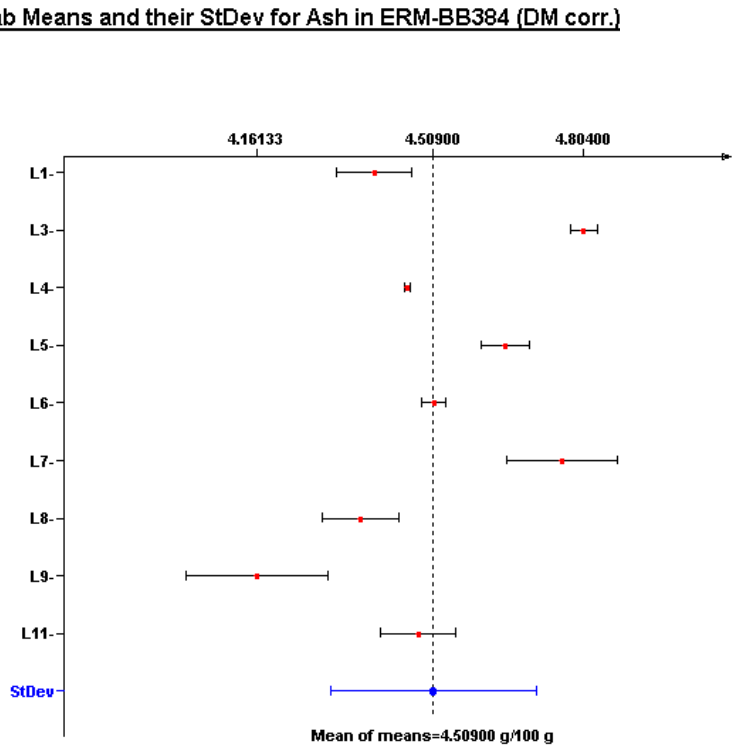


Figure D3: Laboratory means, mean of means and corresponding standard deviations for ash in ERM®-BB384 (related to dry mass)

Table D4: Results of characterisation measurements of sodium content in ERM®-BB384 (related to dry mass)

	Sodium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	1.72	1.71	1.78	1.58	1.64	1.59
3	1.88	1.81	1.80	1.87	1.86	1.88
4	1.61	1.69	1.41	1.59	1.59	1.58
5	1.79	1.79	1.79	1.78	1.84	1.82
6	2.18	2.22	2.05	2.19	2.12	2.09
7	2.01	2.07	2.00	1.90	1.89	1.88
8	1.91	1.92	1.88	1.91	1.91	1.83
9	1.66	1.75	1.67	1.72	1.73	1.72
10	1.87	1.85	1.85	1.87	1.85	1.84
11	2.00	2.05	2.22	2.14	2.24	2.17

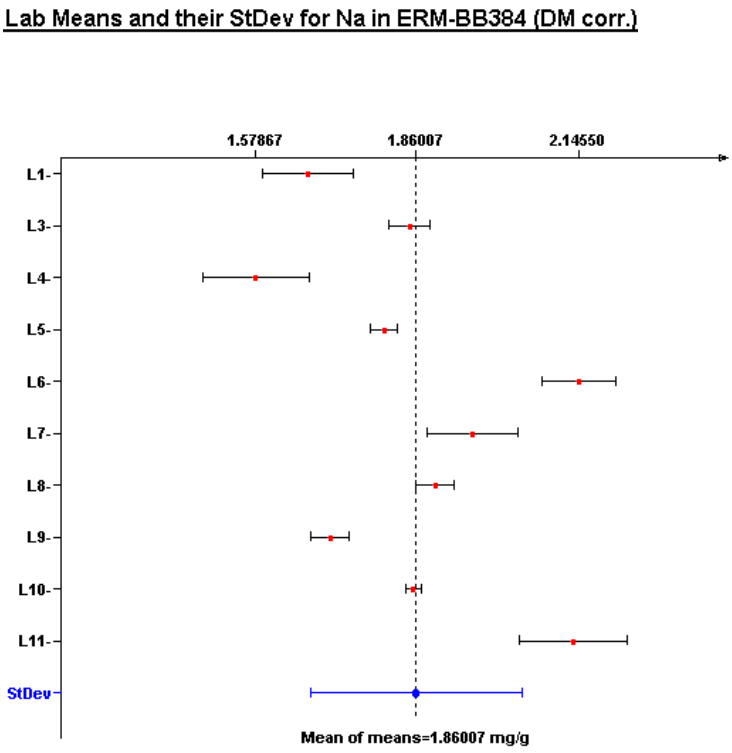


Figure D4: Laboratory means, mean of means and corresponding standard deviations for sodium in ERM®-BB384 (related to dry mass)

Table D5: Results of characterisation measurements of magnesium content in ERM®-BB384 (related to dry mass)

Lab code	Magnesium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]					
	Day 1			Day 2		
1	1.02	0.96	0.98	1.03	1.04	1.03
3	0.96	0.96	0.92	1.03	0.99	1.01
5	1.01	1.00	1.00	0.99	1.03	1.03
6	1.05	1.00	1.01	1.01	1.03	1.02
7	1.03	0.99	1.02	1.02	1.02	1.01
8	1.08	1.07	1.04	1.05	1.05	1.02
9	1.04	1.03	1.04	1.03	1.05	1.03
10	1.06	1.05	1.06	1.04	1.06	1.05
11	1.07	1.11	1.11	1.08	1.08	1.06

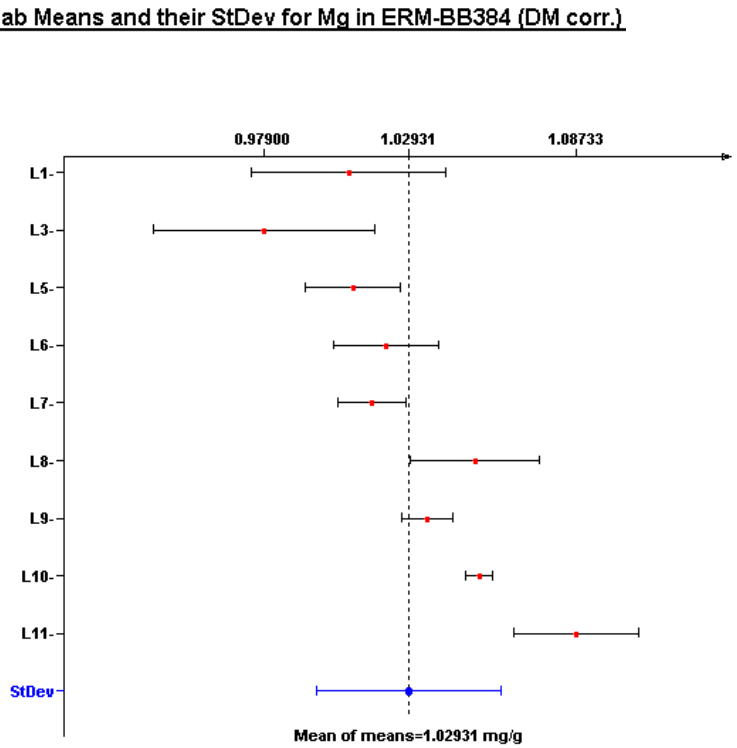


Figure D5: Laboratory means, mean of means and corresponding standard deviations for magnesium in ERM®-BB384 (related to dry mass)

Table D6: Results of characterisation measurements of calcium content in ERM®-BB384 (related to dry mass)

	Calcium mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	0.14	0.15	0.16	0.13	0.13	0.13
3	0.15	0.14	0.17	0.14	0.13	0.13
4	0.14	0.14	0.12	0.14	0.14	0.15
5	0.15	0.15	0.15	0.15	0.17	0.16
7	0.16	0.16	0.16	0.16	0.16	0.17
8	0.18	0.27	0.17	0.18	0.21	0.19
10	0.22	0.21	0.18	0.19	0.16	0.17
11	0.17	0.18	0.17	0.18	0.20	0.20

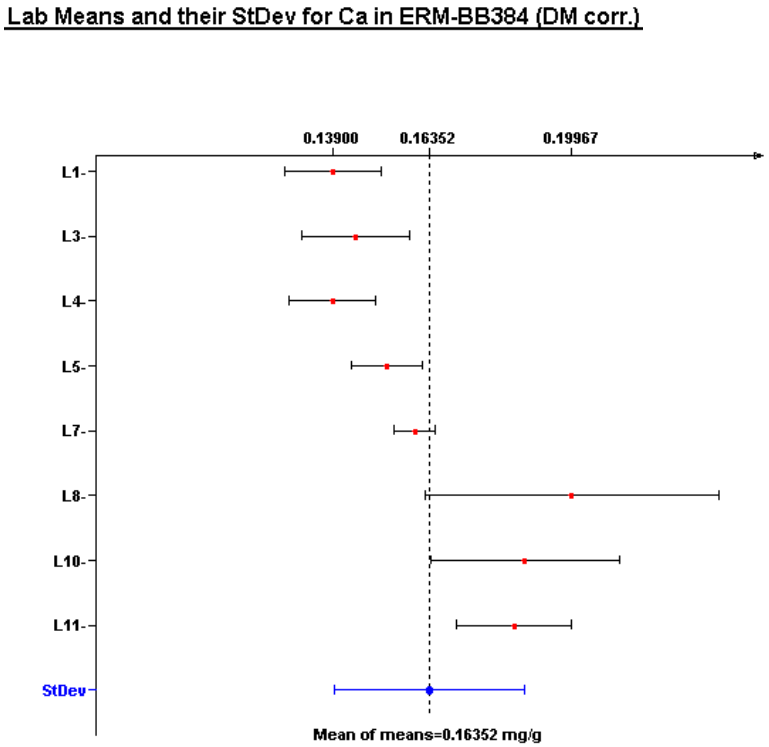


Figure D6: Laboratory means, mean of means and corresponding standard deviations for calcium in ERM®-BB384 (related to dry mass)

Table D7: Results of characterisation measurements of phosphorus content in ERM®-BB384 (related to dry mass)

	Phosphorus mass fraction in ERM®-BB384 (lyophilised pork muscle) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	8.49	8.58	8.44	8.45	8.63	8.30
3	8.66	8.70	8.58	8.59	8.68	8.56
5	9.07	8.95	8.95	9.10	9.20	9.22
6	8.75	8.71	8.68	8.74	8.68	8.64
7	8.50	8.19	8.44	8.41	8.47	8.34
8	8.93	8.92	8.80	8.81	8.94	8.54
9	8.81	8.91	8.85	8.81	8.81	8.85
10	8.18	8.23	8.19	8.20	8.23	8.19
11	9.09	9.23	9.15	9.35	9.40	9.31

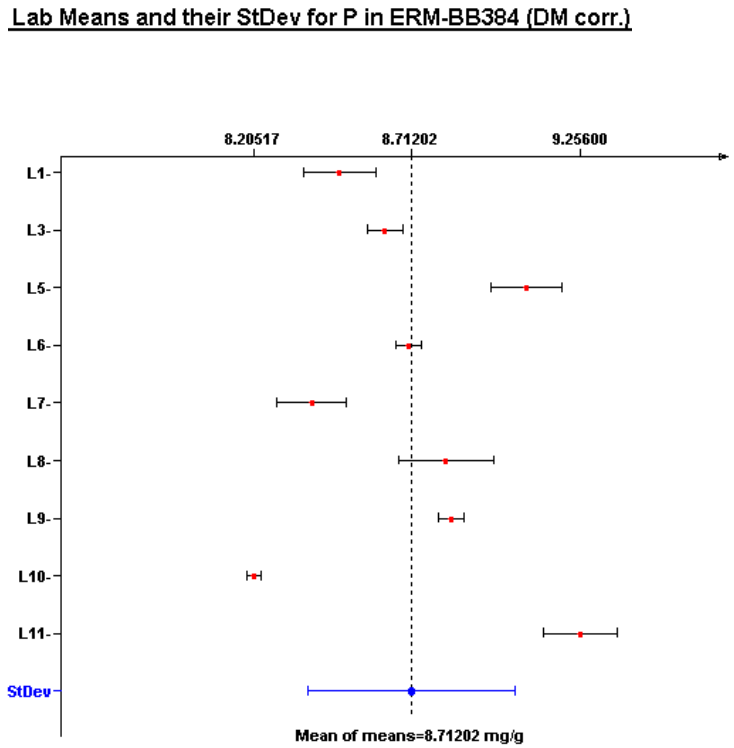


Figure D7: Laboratory means, mean of means and corresponding standard deviations for phosphorus in ERM®-BB384 (related to dry mass)

European Commission

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Title: The Certification of the Mass Fractions of Proximates and Essential Elements in Lyophilised Pork Muscle - Certified Reference Material ERM®-BB384
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Abstract
This report describes the preparation of the lyophilised pork muscle matrix reference material ERM®-BB384 and the certification of the contents (mass fractions) of three proximates and four essential elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the materials, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation. The certified values and their uncertainties are listed in Table I:

Table I: Certified mass fractions of proximates and essential elements and their uncertainties in lyophilised pork muscle (ERM®-BB384)

Proximates and essential elements	Certified value [1)]	Uncertainty [2)]	Number of accepted sets of results
Kjeldahl nitrogen [3)]	14.2 g/100 g	0.4 g/100 g	9
Total fat [4)]	8.99 g/100 g	0.20 g/100 g	10
Ash [5)]	4.51 g/100 g	0.19 g/100 g	9
Na	1.86 mg/g	0.15 mg/g	10
Mg	1.03 mg/g	0.04 mg/g	9
Ca	0.164 mg/g	0.021 mg/g	8
P	8.7 mg/g	0.5 mg/g	9

1) These values are related to dry mass and are based on the unweighted mean of accepted results
2) The uncertainties are the expanded uncertainties ($k = 2$) of the certified values
3) Protein can be derived by multiplying Kjeldahl nitrogen with an appropriate factor (e.g. see ISO 937 [2])
4) Total fat determined after acid hydrolysis, solvent extraction and subsequent gravimetry
5) Ashing at 550 °C ± 25 °C

The assigned values and their uncertainties are based on minimum sample intakes varying from 2 g each for dry mass, total fat and ash, 1 g each for sodium, magnesium, calcium and phosphorus and 0.5 g for Kjeldahl nitrogen.

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